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Human neural progenitor cells have a strong potential for use as cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural cell lines. In this report, we detail progress in development for the following areas: (1) neural progenitor isolation from induced pluripotent stem cells, (2) directed differentiation of progenitors into dopaminergic neurons and astrocytes and (3) HTS amenable assays for proliferation, differentiation, cell migration, mitochondrial function, reactive oxygen species generation and apoptosis as sensor elements.

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425 River Road
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Quarterly Report
Human Neural Cell-Based Biosensor

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Summary

Human neural progenitor cells have a strong potential for use as cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural cell lines. In this report, we detail progress in development for the following areas: (1) neural progenitor isolation from induced pluripotent stem cells, (2) directed differentiation of progenitors into dopaminergic neurons and astrocytes and (3) HTS amenable assays for proliferation, differentiation, cell migration, mitochondrial function, reactive oxygen species generation and apoptosis as sensor elements.

(1) Neural progenitor isolation from induced pluripotent stem cells (iPSCs)

Using our previously developed methods to generate iPSCs and isolate neural progenitor cells, we have generated a new iPSC-derived neural progenitor line. This new line has been amplified into a working stock for beta testing and potential commercial distribution. We are currently characterizing this progenitor working stock and using our proprietary differentiation method to differentiate them toward more mature neuronal populations. We are also deciding on beta test sites, developing quality control standards and optimizing conditions for production runs.

(2) Directed differentiation

We are continuing development of methods for directed differentiation of neural progenitors into dopaminergic and astrocytes. Substantial progress has been made with astrocyte differentiation procedure; recent work optimizing conditions to robustly and reliably differentiate hNP1™ cells has been presented in poster format at the International Society for Stem Cell Research 9th Annual Meeting, June 15 - 18, 2011, Toronto, Canada, and at the Society for Neuroscience Annual Meeting, November 12-16, 2011, Washington, D.C. The manuscript of these findings on astrocyte differentiation is currently being written for future publication. Work also continues on optimizing the differentiation protocols for hNP1™ derived dopaminergic neurons. We have continued to optimize media formulations. In addition we have also begun exploring transient genetic modification strategies to increase our yields of dopaminergic neurons.

(3) Development of HTS amenable assays as sensor elements for neurotoxicity

We continue to make rapid, substantial progress in developing fluorescence based assays as sensor elements in cell-based biosensors.

Alamar Blue and Cellular ATP assays: We have optimized conditions for using both hNP1™ and hN2™ cells with the Alamar Blue and Cellular ATP assays. We intend to use these assays as to measure proliferation and cellular metabolism. We have begun testing known inducers of apoptosis and mediators of neuronal toxicity to complete proof-of-concept studies for these assays.

Neurite outgrowth assay: We have continued to make substantial progress in our development of a neurite outgrowth assay using both our proliferative neural progenitor hNP1™ cell line and our differentiated, mixed neuronal hN2™ cell line with an ImageXpress® high content imaging platform. This assay allowed for the detection of neurotoxicants as well as positive and negative modulators of neuronal differentiation, with output parameters including the number of neurites, length, branches, etc. per cell or per field. Our progress was recently presented in poster format at the Society for Neuroscience Annual Meeting, November 12-16, 2011, Washington, D.C. We are in the process of marketing our hNP1™ and hN2™ cell lines with high content imaging platforms as a screening tool for drug discovery and toxicology studies.

Cell migration assay: We have continued our development of an HTS-amenable cell migration assay using hNP1™ neural progenitor cells to identify inhibitors and stimulators of migration. We have presented our progress to date in poster format at the International Society for Stem Cell Research 9th Annual Meeting, June 15 - 18, 2011, Toronto, Canada, and at the Society for Neuroscience Annual Meeting, November 12-16, 2011, Washington, D.C.